Genetic Diversity of Cultivated and wild-Type potatoes under *Potato Spindle Tuber Viroid* Infection

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Abstract: Potato cultivars and wild species are varying diversity in their response to PSTVd infection. It was found that, all the cultivated potatoes were susceptible to PSTVd infection except (Draga and Spunta), (Hermes) and (Nicola) were tolerant, hypersensitive and low resistant, respectively. On the other hand, all wild species were tolerant except *S. etuberosum* was immune and *S. chacoense* was rather moderate resistant. Five of the RAPD and four ISSR primers were used to detect DNA markers for PSTVd-resistant among cultivated and wild potato species. These primers revealed polymorphism depending on bands number and level of detectable polymorphism of primers. Thus, the hypersensitive plants of Hermes appeared (10), the tolerant plants of Draga showed (8) markers with both of RAPD and ISSR, and the resistant plants of Nicola revealed (3) one marker by RAPD and two markers by ISSR. Also, the immune plants of *S. etuberosum* scored the highest markers for resistant plants to PSTVd (11) markers (one in RAPD and 10 with ISSR), then the resistant plants *S. chacoense* displayed (1) with RAPD.

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1. Introduction

Potato Spindle Tuber Viroid (PSTVd) is the type member of the genus *Pospiviroid* (Family *Pospiviroidae*). It is a circular single-stranded, RNA molecule, measuring between 356-361 nt in length and un-encapsidated (Schnöelzer et al., 1985; Wang et al*.,* 2011). The wild-type potato inoculated with PSTVd such as *Solanum acaule, S. chacoense* and *S. demissum* gave rosette like growth and necrosis of stem petioles or leaves, *S. berthaultii* showing leaf drop, necrosis and necrotic lesions. Whenever, *S. guerreroense* and *S. stoloniferum* showed rosette-like growth, stunted stem and leaves stunted (small leaf diameter) (Singh and Slack 1984). Viroids provide a minimal genetic and biochemical system for the study of the mechanisms controlling host-pathogen interactions and the control of gene expression in plant cells. PSTVd accumulates in the nuclei of infected cells and its lack of mRNA activity suggests that viroids alter host metabolism by direct interaction with one or more host components. Although, viroid infection is not accompanied by gross changes in nucleic acid metabolism, the concentration of various host proteins may change markedly**.** Increased transcription of defense-related genes has been observed in viroid-infected plants and can be mimicked by the application of ethephon, an ethylene-releasing compound (Harders et al., 1989 and Gadea et al., 1996). The molecular mechanisms of viroid pathogenicity, as well as host response are poorly understood. Molecular genetic markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), intersimple sequence repeats (ISSR) and simple sequence repeats (SSR) have been used extensively in genetic studies of potato cultivars. (Granell et al., 1987; Chani, 1997; Itaya et al., 2002).

2. Materials and Methods

Potato cultivars

Eight potato cultivars were obtained from (Ministry of Agriculture, Dokki, Giza, Egypt) (Table 3). These cultivars were tested against potato viruses (*Potato Virus X*, *Potato Virus Y* and *Potato Leaf Roll Virus*) using DAS-ELISA (Clark and Adams 1977). Thirteen tubers were virus-tested from each cultivar were planted in Grower mix (Peatmoss pH 5.6 original formula avec perlite-Canada) in black plastic bags (35 x 27 cm). The seedlings Potato at five-to-six-leaf stage from each cultivar were mechanically inoculated with PSTVd isolate. The inoculated and control plants were grown in greenhouse at 25ºC with a photoperiod 16 h. Plants were fertilized with a (19-19-19-N-P-K) solution. PSTVd symptoms were recorded on inoculated plants daily for 8 wks. The plants showing distinct symptoms were tested by dot-blot hybridization. Disease index calculated as described by Tian et al, (1985), using 11 grades of symptoms.

Disease index =

(Number of plants of each grade x Disease grade) x 100

Total number of plants x the highest grade

0= No symptoms, 1= Erect, 2= dark green,
3= Twisting, 4= Epinasty, 5= Rugosity,
6= Systemic necrotic spots, 7= Rosette like growth, 8= Leaves stunting, 9= Stunting,
10= Chlorosis, 11= Leaf drop

Wild-type Potato

True-seed potato species (Table 4) (Centre for Genetic Resources, The Netherlands) were tested against potato viruses (PVX, PVY and PLRV) using DAS-ELISA. Thirty seedlings from all cultivar were transplanted in Grower mix in pots (14) and were mechanically inoculated with PSTVd isolate. The inoculated plants and control were maintained in insect-proof greenhouse at 18±2ºC supplemented with 16 daylight. The plants were observed daily at two months for visible symptoms. The results of susceptible cultivars were confirmed by Dot-blot hybridization. Disease index calculated as described above.

Molecular markers

DNA extraction

Potato pants immune, resistant, tolerant, hypersensitive and the healthy control were collected and soaked in liquid nitrogen for DNA extraction using the 2% CTAB method modified by (Agrawal et al., 1992). Random amplified polymorphism DNA-polymerase chain reaction (RAPD-PCR) technique.

RAPD-PCR

RAPD is amplification of genomic DNA using five primers as in Table (1) (Kang and Yang 2004). The following components were added to a sterile eppendorof tube placed on ice during pipetting as followed: 2.5 µl 25 mM $MgCl_2$; 0.5 µl 40 mM dNTPs; 1 µl *Taq* DNA polymerase (1 unit/µl); 2 µl 0.4 uM 10-mer primer (manufactured by Bioneer, New technology certification from ATS Korea). The volume was completed to 25 µl dsH_2O.

Thirty nanogram from each DNA extracted sample were used for amplification reaction 5 µl of the 10-mers random primer (15 ng /ml) were added to Gene pack PCR tubes kits. The total volume was completed to 25 µl using sterile dsH_2O. The amplification protocol was carried out as follows using PCR Programe (Biometra): Denaturation at 94ºC for 1 min. 35 cycles each consists of the following steps: Denaturation at 94ºC for 30 sec. annealing at 45ºC for 1 min, extension at 72ºC for 1 min and final extension at 72ºC for 5 min.

ISSR-PCR profiles

A total of four primers Ratnaparkhe et al, (1998) and Bornet et al, (2002) (Table 2; Life Technologies, Gaithersburg, Md.). Each 25-µl amplification reaction consisted of 10X PCR buffer (2.5 µl), 25 mM $MgCl_2$ (2.5 µl), 40 mM dNTPs (0.5 µl), *Taq* DNA polymerase (1 µl, 1 U/µl) and 0.4 µM primer (2 µl). Amplification was carried out in a DNA thermocycler (Biometra, Göttingen, Germany) under the following conditions: One cycle of 3 min at 94°C followed by 28 cycles of 45 s at 94°C, annealing temperature (Table 2) for 30 s and 72°C for 2 min followed by a final extension for 6 min at 72°C.

Table 1. The sequences of used primers were as follows:

Primer	Sequence
Primer-1	TCGCTCGTT
Primer-2	AAC GCG CAA C
Primer-3	CCC GTC AGC A
Primer-4	CCA CAG CAG T
Primer-5	AAG CCC GAG G

Table 2. Sequences and annealing temperatures of the five primers ISSR selected to amplify DNA from potato leaves.

Primer	Sequence	TA(ºC)
HB08	$(GAG)_2$ $(AGA)_2$ GG	44
Primer-1	GAG $(CAA)_5$	55
Primer-3	CTG $(AG)_8$	55
Primer-11	$(AG)_8$	49

Amplification product analysis

The amplified DNA (15 µl) for all samples was electrophoresed on 1% agarose containing ethedium bromide (0.5 µg/ml) in 1x TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) at 75 constant volt, and determine with UV transilluminator. The gels were scanned for molecular weight (bp). The different molecular weights of bands were determined against DNA ladder (manufactured by BioRoN) with molecular weights (1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 bp).

Gel analysis

The gel analysis was applied by programme (UVI geltec version 12.4, 1999-2005, USA).

3. Results

Sensitivity of potato cultivars and wild species to PSTVd infection

Potato cultivars

Eight local potato cultivars free from PVY, PVX and PLRV were tested for sensitivity to PSTVd infection. It was found that, all potato cultivars were

susceptible to PSTVd isolate and no cultivar was resistant. Susceptible reactions of individual plants in eight cultivars ranged from hypersensitivity such as cv. Hermes (55 disease index), tolerant such Draga and Spunta (0 and 12.5 disease index) respectively and susceptible such as: Burren; Cara; Diamond and Lady Rosetta (60.41; 62.50; 46.66 and 100 disease index, respectively). Moreover, Nicola was low resistant, thus eight plants from twelve of cv. Nicola was resistant to infection with PSTVd-isolate (32.22 disease index). These results were confirmed by NASH (Table 3).

Wild species potato

Sensitivity of seven wild species potato free from PVY, PVX and PLRV to PSTVd-EG infection was studied. It is found that, cv. *Solanum demissum* clone 17783 gave hypersensitivity (58.33 disease index), whereas *S. acule* 17926; *S. berthaultii* 18074, *S. guerreroense* 18290 and *S. stoloniferum* 18333 were tolerant and *S. chacoense* 17901 was rather moderate resistant (16.67 disease index). Whenever, *S. etuberosum* 17714 was immune to infection with PSTVd isolate. These results were confirmed by NASH (Table 4).

Molecular marker of potato cultivars and wild species under PSTVd-infection

RAPD-PCR

Total numbers of 87 scorable amplified DNA fragments ranging in size from 2090 to 105 bp were observed using the five primers, whereas 58 fragments were polymorphic and the other amplified fragments were monomorphic detected among the three potato cultivars under PSTVd infection (Table 5). The five primers; 1, 2, 3, 4 and 5 showed mean polymorphic percentage of 66%. The polymorphic percentage of primer-3 recorded the highest percentage (73.3%), whereas primer-4 displayed the lowest percentage (57.1%). The other three primers; 1, 2 and 5 showed the polymorphic percentages 70.6, 66.7 and 65% respectively.

Among the 58 polymorphic bands, nine bands were specific markers to the resistance for PSTVd with a total average of 10.2%. The three cultivars were varied considerably in their resistant markers using the five primers, whereas the hypersensitive plants of Hermes revealed the highest number with five markers, followed by the tolerant plants of Draga with four markers, while the resistant plants of Nicola cultivar revealed one marker (Table 5).

On the other hand, a total number of 55 scorable amplified DNA fragments ranging in size from 1300 to 100 bp were observed using the five primers, whereas 47 fragments were polymorphic and the other amplified fragments were commonly detected among the two potato wild types under PSTVd infection (Table 6). The five primers; 1, 2, 3, 4 and 5 showed mean polymorphic percentage 85.5%, whereas the polymorphic percentage of primer 1 and 4 recorded the highest percentage (75%), while primer-5 displayed the lowest percentage (66.7%). Primer-2 showed the polymorphic percentage was 72.7%, while the primer-3 showed (73.3%).

Among the 47 polymorphic bands, two bands were specific markers to the resistance for PSTVd with a total average of 3.6%. Besides, the two-types were varied considerably in their resistant markers using the five primers, whereas both of the immune plants of *S. etuberosum* and the resistant plants of *S. chacoense* revealed one marker (Table 6).

ISSR-PCR

Four random primers; 1, 3, 11 and HB08 were used in ISSR analysis in the uninfected and PSTVd infected plants of three potato cultivars and two wild species.

The results summarized in Table (7) showed that a total number of 47 scorable amplified DNA fragments ranging in size from 1585 to 100 bp were observed using the four primers, whereas 38 fragments were polymorphic. The four primers; 1, 3, 11 and HB-08 showed mean polymorphic percentage of 83%, whereas the polymorphic percentage of primer HB-08 recorded the highest percentage (100%), while primer-11 displayed the lowest percentage (66.7%). The primers; 1 and 3 showed the polymorphic percentages were 71.4 and 77.8% respectively.

Among the 38 polymorphic bands, 11 bands were specific markers to the resistance for PSTVd with a total average of 23.4%. The hypersensitive plants of Hermes appeared the highest number with five markers, followed by the tolerant plants of Draga revealed four. Finally, the resistant plants of Nicola cultivar with two markers (Table 7).

A total of 50 scorable amplified DNA fragments ranging in size from 1290 to 100 bp were observed using the four primers, whereas 40 fragments were polymorphic and the other were monomorphic detected among the two *Solanium* species under PSTVd infection (Table 7). The four primers; 1, 3, a total average of 20%. The two potato species were varied in their resistant markers using the four primers, whereas the immune plants of *S. etuberosum* revealed the highest number with 10 markers, while the resistant plants of *S. chacoense* showed no markers (Table 8).

Primers 11 and HB08 showed mean polymorphic percentage of 80 %, whereas the polymorphic percentage of primer-1 recorded the highest percentage (92.9%), while primer-3 displayed the

lowest percentage (55.6%). The other two primers 11 and HB08 showed 73.3 and 91.7% respectively.

Among the 40 polymorphic bands, 10 bands were specific markers to the resistance for PSTVd The results recorded in Table (9) it was obtained that an overall spectrum of resistant markers to PSTVd infection. The two different markers techniques RAPD and ISSR were combined together as shown in table (9) the obtained results of three techniques revealed a total of 33 markers, whereas Hermes recorded the highest markers for resistant to PSTVd (10), followed by Draga with (8). However, Nicola displayed the lowest total number of markers with (3). The polymorphic Percentage of the three former cultivars was 47.62, 38.10 and 14.29, respectively.

On the other hand, *S. etuberosum* appeared the highest markers for resistant to PSTVd (11) markers, then *S. chacoense* with one marker. The polymorphic percentages of the three former cultivars were 91.67 and 8.33 respectively.

Table 3. Disease severity of PSTVd-isolate in potato cultivars.

Cultivar	Symptoms	Infectivity assay*	Period of symptoms (days)	Disease index (%)	Confirmed index (NASH)**	Type of resistant
Burren	T,R,LS,Ch	12/12	25-35	60.41	+	Susceptible
Cara	R,LS	12/12	25-42	62.50	+	Susceptible
Diamond	E,LS,R,T,S	12/12	25-31	46.66	+	Susceptible
Draga	NS	12/12	-	Carrier (o)	+	Tolerant
Hermes	E,DG,SNS,Ld,Ch	12/12	25-31	55	+	Hypersensitivity
Lady Rosetta	R,T,LS,EP,Ch	12/12	25-39	100	+	Susceptible
Nicola	T,E,Ch,Ep,LS	8/12	25-39	32.22	+	Low resistant
Spunta	E,DG	12/12	25	12.5	+	Tolerant

*No of infected /No. of inoculated plants.

NASH** = Nucleic acid spot hybridization

Ch = Chlorosis, LS = Leaf stunting (small leaf diameter), Ld = Leaf drop, DG = Dark green, NS = No symptoms, R = Rugosity, E = Erect, R = Rosette like growth, SNS = Systemic necrotic spots, EP = Epinasty, S = Stunting, T= Twisting of terminal leaflets

Table 4. Disease severity of PSTVd in the wild species potatoes.

Cultivar	Symptoms	Infectivity assay	Period of examination (days)	Disease index (%)	Confirmed index (NASH)**	Type of resistant
Solanium acule 17926	NS	30/30	-	0	+	Tolerant
S. berthaultii 18074	NS	30/30	-	0	+	Tolerant
S. chacoense 17901	DG	10/30		16.67	+	Rather moderate resistant
S. demissum 17783	SNS	30/30	29	58.33	+	Hypersensitivity
S. etuberosum 17714	NS	0/30	-	0	-	Immune
S. guerreroense 18290	Ns	30/30	-	0	+	Tolerant
S. stoloniferum 18333	NS	30/30	-	0	+	Tolerant

DG = Dark green, NS = No symptoms, SNS = Systemic necrotic spots, NASH** = Nucleic acid spot hybridization

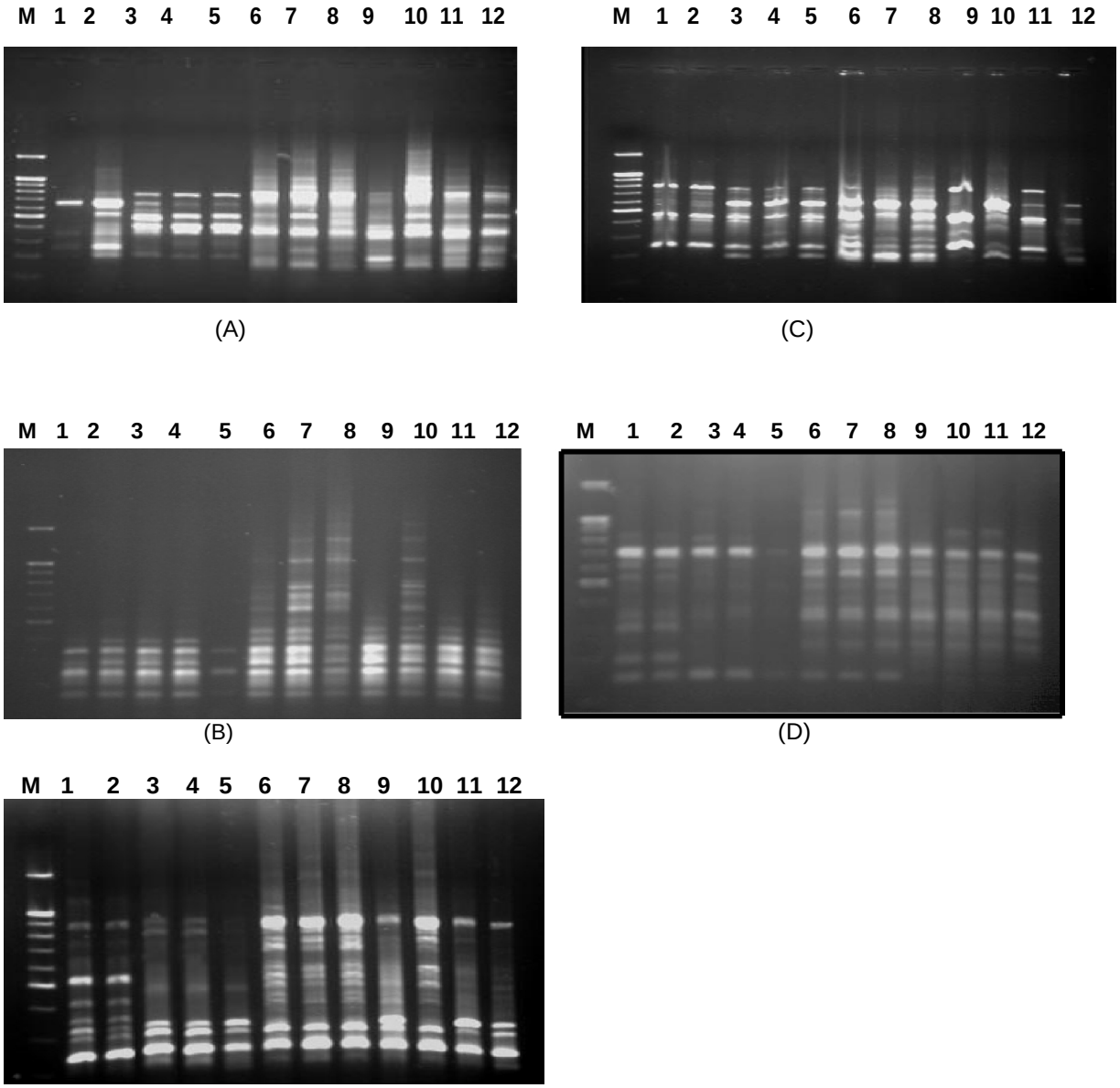

Figure 1. RAPD analysis of potato cultivas and wild species under PSTVd- infection. Lane M = 100 bp DNA ladder. (A) Primer-1,: (B) primer-2, (C) primer-3, (D) primer-4 and (E) primer-5.
1,2=*S. etuberosu*m plants immune for PSTVd infection and the healthy control, respectively.
3,4,5=*S. chacoens*e plants susceptible, resistant for PSTVd infection and the healthy control, respectively. 6,7,8=Nicola plants susceptible, resistant for PSTVd infection and the healthy control, respectively. 9,10=Draga plants tolerant for PSTVd infection and the healthy control, respectively.
10,12=Hermes plants hypersensitive for PSTVd infection and the healthy control, respectively.

Table 5. RAPD amplified bands, polymorphic bands and markers for resistance to PSTVd using five random primers in three potato cultiv

Primer name	Polymorphism		No. of markers with size (bp)		Distribution of RAPD markers		
	Total	P (%)			Nicola	Draga	Hermes
1	17	12	1	1200	-	-	+
	70.6%		5.9%		0	0	1
2	21	14	1	105	-	+	-
	66.7%		4.8%		0	1	0
3	15	11	4	950		+	
				850		+	+
				340		+	
				325			+
	73.3%		26.7%		0	3	2
4	14	8	2	606			+
				177			+
	57.1%		14.3%		0	0	2
5	20	13	1	395	+		
	65%		5%		1	0	0
Total	87	58	9	10.2%	1	4	5
Polymorphic =66%							

* P = Number of polymorphic bands with polymorphic percentages.
** Total = Total number of amplified fragments.
+ = Presence of marker band.

4. Discussion

Eight potato cultivars were tested for response to PSTVd infection. All cultivated potatoes were susceptible to PSTVd infection based on disease index. Response of potato cultivars were ranged from hypersensitive such as Hermes; tolerant (Draga and Spunta) and susceptible (Cara; Diamond and Lady Rosetta) cultivars. As well as, Nicola cultivar was low resistant for PSTVd. In addition, seven wild species of potatoes were tested for response to PSTVd infection. It was found that, *Solanum demissum* gave hypersensitive, whereas *S. acule*; *S. berthaultii*; *S. guerreroense* and *S. stoloniferum* were tolerant, whenever *S. etuberosum* and *S. chacoense* 17901 was immune and rather moderate resistant, respectively to PSTVd infection depend on results of disease index. These results were confirmed by dot-blot hybridization. Many authers (Singh and Slack 1984) reported that the potato cultivars were susceptible and tolerant to PSTVd-s (severe strain) and no cultivars were immune. As mentioned that disease severity caused by PSTVd-s ranged from mild to severe depending upon the potato cultivar and strain of PSTVd. Moreover**,** Allam et al., (1989) found that potato cultivars differed in response to PSTVd infection, whereas disease severity caused by PSTVd-s ranged from mild to severe depended upon the potato cultivar. Alpha cultivar was susceptible, Spunta and Kennebic were mediate and Diamond was tolerant. In the first season infection cultivars developed milder symptoms than second and third seasons infections and tolerant cultivars were less affected than susceptible cultivars. Singh (1985) found that two clone of *S. berthaultii* were resistant to mild and severe strains of PSTVd. Salazar et al., (1988) detected R_{Pacl} gene in *S. acaule* responsible on extreme resistant to PSTVd.

Zaitlin and Hariharasubramanian (1972) and El-Dougdoug (1988) reported that viroid infection does not cause any major qualitative or quantitative changes in the nucleic acid profiles of the host, which would indicate a greater coordination of viroid and host nucleic acid synthesis. Nevertheless, some viroid-induced changes in host proteins have been demonstrated for different viroid-host combinations. A comprehensive analysis of gene expression patterns in viroid-infected plants showed further expand our knowledge of host responses to viroid infection. Furthermore, a comparative analysis of host gene expression patterns during viroid and virus infections may enhance understanding of common and unique genes host response to different pathogens. Also, they found that severe strain of PSTVd altered expression of a total of 52 genes. Among these genes 27 were altered only by severe strain of PSTVd and 10 were also altered by mild strain of PSTVd. Notably two genes were specifically induced by mild PSTVd infection. These include a gene homologous to a unknown gene from *Medicago sativa* (BM556730) was notable, not only because it was specifically induced by PSTVd-m (mild strain) infection, but also because it was induced as carly as five days post-inoculation when most genes were not induced or suppressed by PSTVd-infection. Five genes were induced by PSTVd-s at the late stages of infection. Induction of this genes by viroid infection was reported previous by (Tornero et al., 1994).

Table 6. RAPD polymorphism using five random primers, number of resistant markers to PSTVd and distribution of markers in two potato wild species.

Primer name	Polymorphism		No. of markers with size (bp)		Distribution of RAPD markers	
	Total	P (%)			*S. etuberosum*	*S. chacoense*
1	12	9	-	-	-	-
	75%		0%		0	0
2	11	8	-	-	-	-
	72.7%		0%		0	0
3	15	11	1	700	-	+
	73.3%		6.7%		0	1
4	12	9	-	-	-	-
	75%		0%		0	0
5	15	10	1	1230	+	-
	66.7%		6.7%		1	0
Total	55	47	2		1	1
Polymorphic = 85. 5%			3.6%			

* P = Number of polymorphic bands with polymorphic percentages.
** Total = Total number of amplified fragments. + = Presence of marker band.

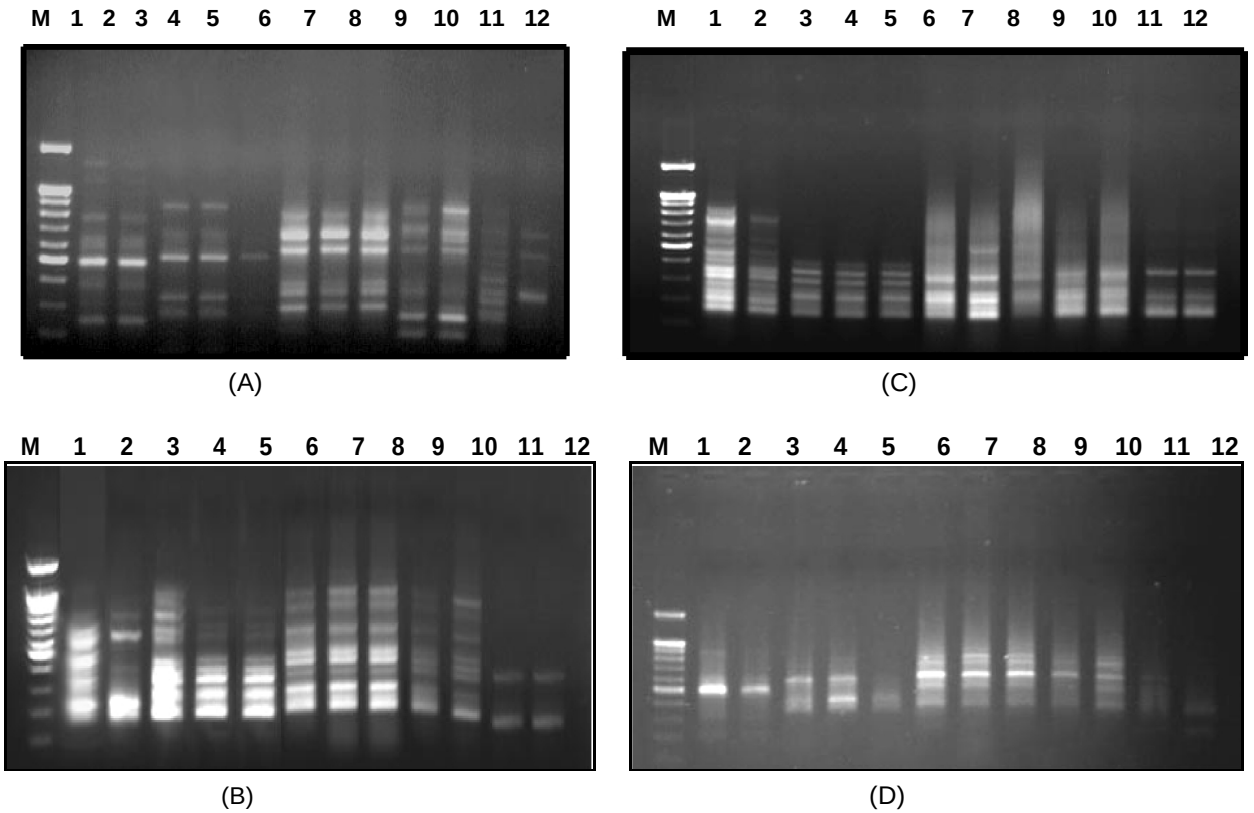

Figure 2. ISSR analysis of potato cultivas and wild species under PSTVd- infection.
Lane M = 100 bp DNA ladder. (A) Primer-1: (B) primer-3, (C) primer-11 and (D) primer- HB08.
1,2= *S. etuberosu*m plants immune for PSTVd infection and the healthy control, respectively.
3,4,5= *S. chacoens*e plants susceptible, resistant for PSTVd infection and the healthy control, respectively. 6,7,8=Nicola plants susceptible, resistant for PSTVd infection and the healthy control, respectively. 9,10=Draga plants tolerant for PSTVd infection and the healthy control, respectively.
10,12=Hermes plants hypersensitive for PSTVd infection and the healthy control, respectively.

Table 7. ISSR amplified bands, polymorphic bands and markers for resistance to PSTVd using four random primers in three potato cultivars.

Primer name	Polymorphism		No. of markers with size (bp)		Distribution of ISSR markers		
	Total	P (%)			Nicola	Draga	Hermes
1	14	10	4	975		+	
				740		+	
				475			+
				375			+
	71.4%		28.6%		0	2	2
3	9	7	0				
	77.8%		0%		0	0	0
11	9	6	1	635		+	
	66.7%		11.1%		0	1	0
HB-08	15	15	6	1585		+	
				1160	+		
				565			+
				460			+
				335	+		
				270			+
	100%		40%		2	1	3
Total	47	38	11	23.4%	2	4	5
Polymorphic = 80.9%							

* P = Number of polymorphic bands with polymorphic percentages.
** Total = Total number of amplified fragments. + = Presence of marker band.

Table 8. ISSR amplified bands, polymorphic bands and markers for resistance to PSTVd using four random primers in two wild species.

Primer name	Polymorphism		No. of markers with size (bp)		Distribution of ISSR markers	
	Total	P (%)			*S. etuberosum*	*S. chacoense*
1	14	13	0			
	92.9%		0%		0	0
3	9	5	3	1077	+	
				590	+	
				345	+	
	55.6%		33.3%		3	0
11	15	11	4	1175	+	
				1036	+	
				635	+	
				290	+	
	73.3%		26.7%		4	0
HB-08	12	11	3	1030	+	
				800	+	
				680	+	
	91.7%		25%		3	0
Total	50	40	10	20%	10	0
Polymorphic = 80%						

* P = Number of polymorphic bands with polymorphic percentages.
** Total = Total number of amplified fragments. + = Presence of marker band.

Table 9. Genetic markers in potato cultivars and wild species resistance for PSTVd.

Molecular markers	RAPD & ISSR Primers	Potato cultivars				Potato wild species		
		Nicola	Draga	Hermes	Total	*S. etuberosum*	*S. chacoense*	Total
RAPD-PCR	1	0	0	1		0	0	
	2	0	1	0		0	0	
	3	0	3	2		0	1	
	4	0	0	2		0	0	
	5	1	0	0		1	0	
	Total	1	4	5	10	1	1	2
ISSR-PCR	1	0	2	2		0	0	
	3	0	0	0		3	0	
	11	0	1	0		4	0	
	HB-08	2	1	3		3	0	
	Total	2	4	5	11	10	0	10
	Overall no.=	3	8	10	21	11	1	12
	Polymorphic %	14.29	38.10	47.62		91.67	8.33	

In the present study, three different marker techniques, protein (gene expression), RAPD and
ISSR were used to characterize some cultivars and wild species under PSTVd infection and to detect the resistant markers that could be linked to some resistant genes in potato.

However, no many informative reports could be obtained that establishing any type of PCR markers neither in potato cultivars nor in wild species under PSTVd infection. Consequently, such topics are not well documented yet and this study is the first record in potato.

Five of the RAPD and four from ISSR primers were used to find markers for PSTVd-resistant cultivated and wild potatoes. These primers revealed polymorphism depending on bands number and level of detectable polymorphism. The two different markers techniques (RAPD and ISSR) were combined together. These results were similar with Bornet et al., (2002) who reported that molecular markers based on PCR amplification are efficient tools, quick and easy for plant breeding programs. These markers include RAPD and ISSR techniques. Also, mentioned that ISSR is not need of prior knowledge of the genome sequence; universal; polymorphous; it is more reproducible than RAPD and used for the genetic diversity study of potato.

Many molecular marker techniques are available today. PCR-based approaches are in demand because of their simplicity and requirement for only small quantities of sample DNA (Heldák et al., 2007). Non-anchored (ISSRs) are arbitrary multiloci markers produced by PCR amplification with a microsatellite primer. They are advantageous because no prior genomic information is required for their use. We found that technique stable across a wide range of PCR parameters. Species tested with 2 tri-nucleotide and 2 tetra-nucleotide primers. Thus, non-anchored ISSR markers are a good choice for DNA fingerprinting. Bornet et al., (2002) reported that ISSR-PCR use to assess genetic diversity between cultivated potatoes (*Solanum tuberosum* subs. *tuberosum*). ISSR technology rapidly reveals high polymorphic fingerprints and thus determines the genetic diversity among potato cultivars.

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